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Branchek et al., Trends Pharmacol. Sci. (2000) 21(3):109-117 REVIEW

Galanin receptor subtypes

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The neuropeptide galanin, which is widely expressed in brain and peripheral tissues, exerts a broad range of physiological effects. Pharmacological studies using peptide analogues have led to speculation about multiple galanin receptor subtypes. Since 1994, a total of three G-protein-coupled receptor (GPCR) subtypes for galanin have been cloned (GAL1, gal2 and gal3). Potent, selective antagonists are yet to be found for any of the cloned receptors. Major challenges in this field include linking the receptor clones with each of the known physiological actions of galanin and evaluating the evidence for additional galanin receptor subtypes.

Galanin is a neuropeptide of 29 amino acids in length (30 amino acids in humans) with a highly conserved N-terminal that is associated with biological activity[1,2]. Galanin is widely distributed in tissues such as the brain, spinal cord and gut[3,4], and can regulate numerous processes including feeding, nociception, nerve regeneration, memory, neuroendocrine release, and gut secretion and contractility. The receptor(s) meditating these actions have been the focus of intense investigation[2-5]. The aim of this review is to examine existing knowledge about galanin receptor subtypes gained from native and cloned receptor systems, highlight discrepancies and discuss implications for future research.

Native galanin receptors

The first binding profiles derived from native systems with galanin and related peptide analogues (Fig. 1) were consistent with multiple receptor subtypes[4,6-8], although definitive proof required cloning. Peptides displaced [^{125}I]galanin binding to human Bowes melanoma cell membranes with a distinctive order of potency: galanin-1–30 > galanin-1–16 > D-Trp^2-galanin ≥ galanin-3–30 (Ref. 9). [^{125}I]galanin binding sites with a difference in affinity of up to a factor of ten for galanin and galanin-1–16 were observed in both the ventral hippocampus[10] and hypothalamus[11]. A site that binds preferentially to the N-terminal of rat [^{125}I]galanin-1–15 has been characterized in rat brain regions including the dorsal hippocampal formation, neocortex and neostriatum[6]. A subtype with affinity for galanin-3–29 has been proposed from binding studies in the rat anterior pituitary and hypothalamus (with an affinity order of: galanin-3–29 > galanin-1–15, M15)[7]. Studies of gastric smooth muscle cells in the guinea-pig have revealed another profile in which rat or porcine galanin-1–29, galanin-1–20, galanin-1–15, galanin-1–10, and also porcine galanin-2–29 and galanin-3–29 have approximately equal affinity[8].

Interactions with multiple signal transduction pathways have resulted in further speculation about receptor subtype diversity in native systems. Galanin not only reduces the concentration of cAMP but also closes voltage-sensitive Ca^{2+} channels and opens ATP-sensitive or inwardly rectifying K^+ channels through $G_{i/o}$-type G proteins[3]. [$G_o\alpha_1\beta_2\gamma_2$ was identified, using antisense techniques, as a preferred heterotrimer for galanin receptors linked to voltage-sensitive Ca^{2+} channels in the rat insulinoma cell line RIN-m5f and in the rat pituitary tumour cell line GH3 (Ref. 12)]. One or more of these pathways and/or the activation of calcineurin[13] or the regulation of an exocytotic event distal to Ca^{2+} entry[3] are thought to underlie the inhibitory effect of galanin on exocytosis. Conversely, galanin also facilitates exocytosis[3] (presumably via Ca^{2+} mobilization), which is probably part of a signalling cascade that involves the activation of phospholipase C (Refs 14, 15), phospholipase A_2 (Ref. 16), mitogen-activated protein (MAP) kinase[17] and mitogenesis[18]. Interestingly, galanin both stimulates and inhibits inositol phospholipid turnover in isolated pancreatic islets[15]; the same is true for Ca^{2+} mobilization in the rat insulinoma cell line RIN-m5f (Ref. 19). Galanin also elevates cAMP accumulation in gastric smooth muscle cells[20].

Diverse pharmacological profiles have also been derived from functional studies in native systems. In Bowes melanoma cells, galanin analogues reduced the concentration of cAMP in the following order of potency: galanin-1–30 > galanin-1–16 > D-Trp^2-galanin ≥ galanin-3–30 (Ref. 9). Chimeric galanin peptides mimic galanin in this respect; galanin-1–30 has a similar potency to M15, M32 and M35, which are more potent than C7 and M40. Different profiles have been obtained within tissues or *in vivo*. For example, when infused into the lateral ventricle, galanin-1–15 is more potent than galanin-1–29 in reducing the sensitivity of the rat baroceptor reflex[21]. The existence of a receptor that is selective for galanin-1–15 is suggested by electrophysiological recordings in the rat dorsal hippocampus[22]. Galanin and galanin-15–29 produce dissimilar effects in the opossum internal anal sphincter, in which the resting tension is reduced by galanin but stimulated by galanin-15–29, and the electric-field-stimulated reduction in the resting tension is enhanced by galanin but unaffected by galanin-15–29 (Ref. 23). Other profiles characterize the release of prolactin from the rat anterior pituitary[7] (potency order: galanin ~ galanin-3–29 > galanin-5–29 > galanin-1–15, M15) and the stimulation of cAMP in gastric smooth muscle[8] (potency order: galanin ~ galanin-3–29 > galanin-9–29 > galanin-21–29). Galanin-3–29 was reported to depress synaptic transmission weakly in the arcuate nucleus of the rat hypothalamus[24].

The use of chimeric peptides (Fig. 1) as tools to characterize galanin receptors in physiological studies (ranging from behavioural to second messenger) has proved to be an interesting and complicated endeavour. For example, M40 has been described as a galanin receptor antagonist in the brain[3,24]

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lative peptides (agonists)	Amino acid position 1 5 10 14 15 20 25 26 30
it, mouse galanin	GWTLNSAGYLLGPH AIDNHRSFSDK HGLT
Human galanin	GWTLNSAGYLLGPH AVGNHRSFSDK NGLTS*
Porcine galanin	GWTLNSAGYLLGPH AIDNHRSFHDK YGLA
Tuna galanin	GWTLNAAGYLLGPH GIDGHRTLGDK PGLA

imeric peptides (proposed antagonists)	Amino acid position 1 5 10 13 14 20 25
M15	GWTLNSAGYLLGP QQFFGLM
C7	GWTLNSAGYLLGP rPKPQQwFwLL
M40	GWTLNSAGYLLGP PPALALA
M35	GWTLNSAGYLLGP PPGFSPFR
M32	GWTLNSAGYLLGP RHYINLITRQRY

Trends in Pharmacological Sciences

1. Peptide analogues used to explore galanin receptor pharmacology include species homologues of anin, and fragments or point mutants thereof. With the exception of tuna fish galanin[1], all other known anin homologues are absolutely conserved in residues 1–14. Human galanin is exceptional in con- ning 30 amino acids rather than 29, and in terminating with a C-terminal free acid (asterisk) rather n an amide. The widely used chimeric peptides share a common motif in which the biologically active erminal region of galanin-1–13 is linked to an amidated C-terminal domain from other neurotrans- ters (e.g. substance P-5–11 for M15, NPY_{25-36} for M32 and bradykinin-2–9 for M35) or a novel sequence . the substance P receptor antagonist spantide for C7 (D-amino acids are shown in lower-case) and a rophobic stretch for M40)[38]. Additional peptide analogues and novel chimerae have been described[39].

and spinal cord[3,25] and a receptor agonist in functional studies of Bowes melanoma cells[9], RIN-m5f cells (Ref. 26), the hypothalamic arcuate nucleus[24] and pancreatic islets[26]. Similarly, M15 has been found to be a receptor antagonist in the hypothalamus[24,27] and pancreas[28], a receptor agonist in functional studies of Bowes melanoma cells[9], gastric smooth muscle[29] and the hypothalamic arcuate nucleus[24], and a partial receptor agonist in RIN-m5f cells[19]; M15 is inactive in the pituitary[7]. The spectrum of agonist or antagonist activity for M40, M15, M35, M32, and C7 (Refs 9, 24, 30–33) has fuelled speculation about multiple receptor subtypes.

Cloned galanin receptors

The GAL1 receptor

The first known galanin receptor GAL1 has been isolated from the human Bowes melanoma cell line[34] and other sources[35,36]. Human GAL1 contains 349 amino acids with the structure of a G-protein-coupled receptor (GPCR)[37]. The highest amino acid similarities are found with human gal2 (42%) and human gal3 (38%) receptors, the rat orphan receptor GPR54 (37%)[38], and human somatostatin and opioid receptors (30–34%)[39]. A rat GAL1 homologue, cloned from brain[10] and RIN-14b cells[39,41], contains 346 amino acids and 92% similarity with GAL1 (Fig. 2). Human and rat GAL1 share the same consensus sites for N-linked glycosylation and for intracellular phosphorylation with the exception of two additional phosphorylation sites in the human GAL1 C-terminal domain. Human GAL1 was mapped to chromosome 18q23 (Ref. 42). The mouse GAL1 homologue has been cloned[43,44] and mapped to chromosome 18E4 (Ref. 43), syntenic with the human gene position.

Human GAL1 mRNA has been detected by northern blot analysis in foetal brain and small intestinal tissue, and in Bowes melanoma cells[34], and also by reverse transcriptase-polymerase chain reaction (RT-PCR) in the human gastrointestinal tract from the oesophagus to the rectum[35]. Rat GAL1 mRNA has been detected by northern blot analysis in the brain, spinal cord and RIN-14b cells[39]. In the CNS, the distribution of rat GAL1 mRNA, determined by *in situ* hybridization, is in good accord with [^{125}I]galanin binding sites and galanin expression: it occurs in the hypothalamus (supraoptic nucleus), amygdala, ventral hippocampus, thalamus, brainstem (medulla oblongata, locus coeruleus and lateral parabrachial nucleus) and spinal cord (dorsal horn)[39–41]. The pattern of GAL1 mRNA expression indicates plasticity. For example, hypothalamic GAL1 mRNA is elevated in females more than males, and varies across the oestrous cycle[45]. In another example, GAL1 mRNA expression in the rat dorsal root ganglia was decreased after inflammation or peripheral nerve injury[46,47]; however, rat GAL1 mRNA was not detected in the facial nucleus either before or after experimental injury of the facial nerve[48]. In other studies, GAL1 mRNA was elevated in hypothalamic nuclei of rats either injected with the glucose anti-metabolite 2-deoxy-D-glucose or the fatty acid anti-metabolite sodium mercaptoacetate, both of which stimulate feeding[49], or following salt loading[50]; conversely, GAL1 mRNA was decreased by lactation and hypophysectomy[50].

Human and rat GAL1 share similar binding profiles in [^{125}I]galanin binding assays (Table 1)[51,52]. The cloned GAL1 receptor reduces the concentration of cAMP (Refs 34, 39, 40, 53), opens G-protein-coupled, inwardly rectifying K^+ channels[51], and stimulates MAP kinase activity[54] in a manner that is sensitive to pertussis toxin; this is consistent with coupling to $G_{i/o}$-type G proteins. The profile of potency for the regulation of cAMP by cloned GAL1 receptors[55] resembles that derived from the Bowes melanoma cell line[9]. Chimeric peptides tested in assays such as inhibition of cAMP accumulation or stimulation of GTPγ^{35}S binding were agonists[9,55], even after elimination of the receptor reserve in the case of human GAL1 expressed heterologously in HEK293 cells[55]. The distribution of GAL1 mRNA or GAL1-like pharmacology in the brain and spinal cord[40,41] as well as in the gut[34,35] and pancreas-derived cells such as RIN-14b (Ref. 39) indicates that GAL1 might act through $G_{i/o}$-type G proteins to inhibit the release of neurotransmitters or hormones into many brain regions, with potential effects on feeding, emotion, memory, nociception, gut secretion and motility. The relatively weak agonist activity of M40 in GAL1 functional assays[55] resembles the weak agonist activity of M40 in inhibiting glucose-stimulated insulin release[26], and suggests a possible role for GAL1 in the regulation of insulin and glucose homeostasis. Interestingly, children with a growth hormone insufficiency phenotype display a common deletion of two megabases from chromosome 18q that results in the absence of the GAL1 (Ref. 56), which suggests a potential role for this receptor in human growth and development.

The gal2 receptor

The second galanin receptor subtype to be cloned gal2 was isolated originally from the rat as an expressed cDNA with a coding region disrupted by an intron just after transmembrane

domain (TM) 3 (Ref. 53). Splicing of the intron restored the open-reading frame to reveal the structure of a GPCR containing 372 amino acids (Fig. 2), including three consensus sites for extracellular N-linked glycosylation and several intracellular phosphorylation sites distinct from GAL1. Rat gal2 shares highest amino acid similarity with rat gal3 (55%) and human gal3 (58%) receptors, and relatively less similarity with rat GAL1 (40%), human GAL1 (40%) or rat GPR54 (38%)[34,38,39,53,57,58]. The cloned human gal2 receptor contains 387 amino acids, 15 more than rat gal2 in the C-terminal, with only 85% similarity to this receptor[52,59,60]. Human gal2 has been mapped to chromosome 17q25.3 (Ref. 60); the mouse gal2 homologue has been cloned and mapped to chromosome 11 (Refs 61, 62).

Unlike GAL1, mRNA encoding rat gal2 is widely distributed in almost all rat tissues examined, including the brain (with highest levels found in the hypothalamus, hippocampus, amygdala and pyriform cortex[63] as well as in the dentate gyrus, mammillary nuclei and cerebellar cortex[64]), and peripheral tissues such as the vas deferens, prostate, uterus, ovary, stomach, large intestine, dorsal root ganglia and pancreas-derived cells (e.g. RIN-m5f)[47,53,57,58,64]. In the rat anterior pituitary, gal2 mRNA has been detected by RNase protection, *in situ* hybridization and RT-PCR, with the latter technique supporting the absence of GAL1 mRNA in this tissue[63]; thus, gal2 might mediate the effects of galanin on pituitary hormone secretion in the rat.

Fathi and co-workers have reported that human gal2 is detectable by RT-PCR in several central and peripheral tissues including the hippocampus, amygdala, pituitary, heart and small intestine but unlike rat gal2, is apparently absent from the hypothalamus[60]. Borowsky and co-workers, using the same technique, have detected human gal2 mRNA in the hippocampus, hypothalamus, kidney, liver, small intestine and retina but not in the cerebral cortex, lung, spleen, stomach or pituitary[52]. It remains to be determined whether the differences between these two reports (particularly in the case of the hypothalamus and pituitary) are a result of variability between human subjects or to technical factors.

Rat GAL1 and gal2 share similar pharmacological profiles in that they possess high affinity for full-length and N-terminal fragments of galanin (i.e. those that contain at least galanin-1–15) and also for chimeric peptides but not for galanin-3–29 (Refs 51, 53, 57, 63, 65). However, rat gal2 is distinguishable from GAL1 because gal2 has a greater affinity for D-Trp^2-galanin[51,53] and galanin-2–29 (Ref. 65). Extension of the N-terminal of galanin by seven residues to galanin-(−7)–29 (a modification that leads to relatively reduced potency in the spinal reflex assay[66]) disrupts binding affinity less for gal2 than the other galanin receptor subtypes. Human gal2 displays a similar [^{125}I]galanin binding profile to rat gal2 (Ref. 59) (Table 1), with the exception that D-Trp^2-galanin binds with relatively reduced affinity[52,59,60].

Activation of gal2 leads to the stimulation of multiple intracellular events. The most commonly reported pathway appears to involve phospholipase C, because gal2 mediates pertussis-toxin-resistant inositol phosphate hydrolysis[53,54,60,63], intracellular Ca^{2+} mobilization[53,60] and Ca^{2+}-dependent Cl^-

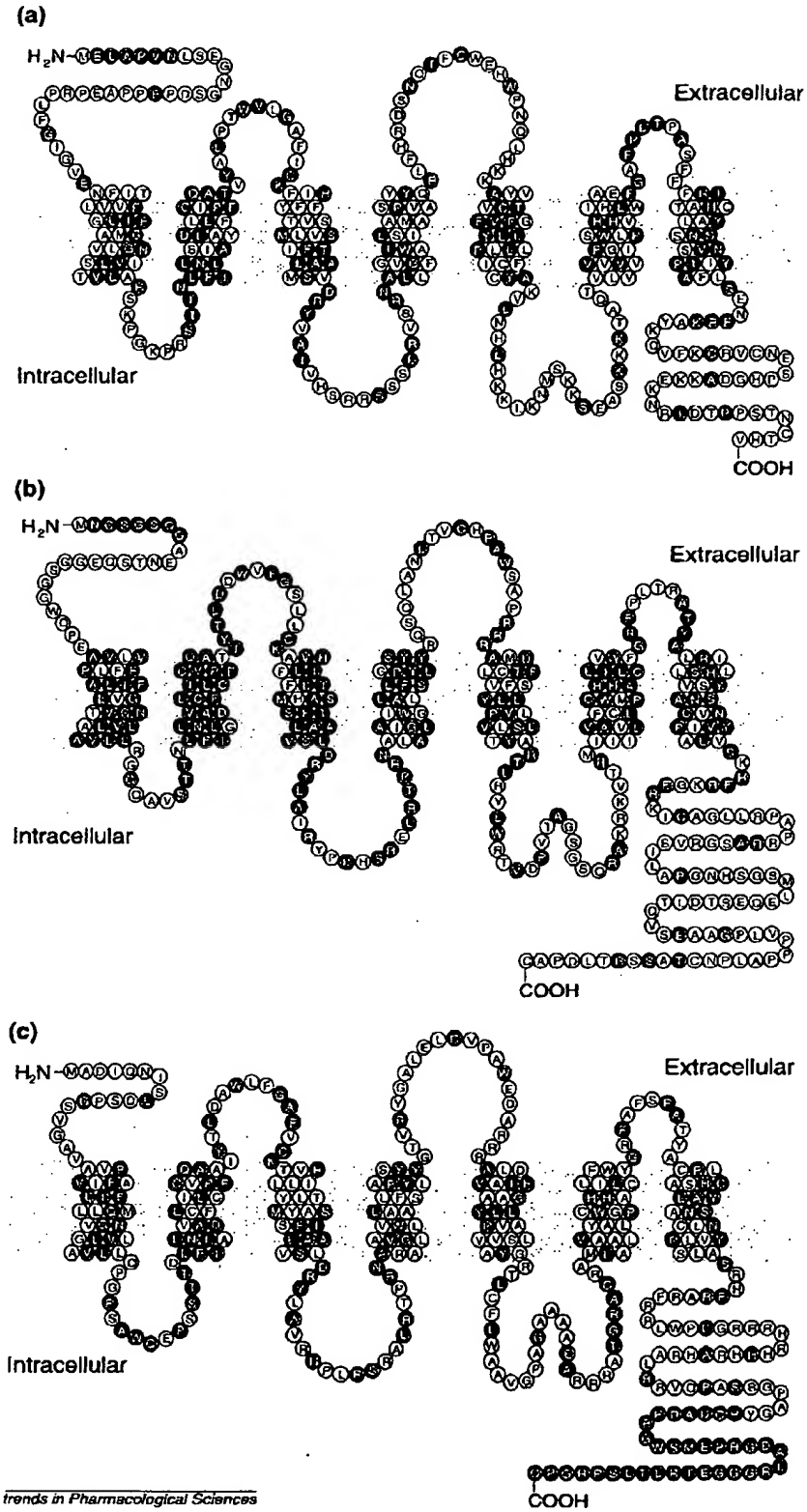

Fig. 2. Schematic diagrams of galanin receptor subtypes cloned from rat. Each subtype is predicted to contain seven transmembrane regions typical of the G-protein-coupled receptor superfamily. **(a)** Rat GAL1 receptor amino acid sequence, coded for similarity to the rat gal2 receptor (blue circles indicate residues that are identical with those in rat gal2; red circles indicate residues that are absent in rat gal2). **(b)** Rat gal2 receptor amino acid sequence, coded for similarity to the rat gal3 receptor (blue circles indicate residues that are identical with those in rat gal3; red circles indicate residues that are absent in rat gal3). **(c)** Rat gal3 receptor sequence, coded for similarity to the rat GAL1 receptor (blue circles indicate residues that are identical with those in rat GAL1; red circles indicate residues that are absent in rat GAL1).

Table 1. Peptide binding: displacement of porcine [^{125}I]galanin from rat and human GAL1, gal2 and gal3 receptors

Compound	pK_i for galanin receptor subtype[a]					
	GAL1		gal2		gal3	
	Human	Rat	Human	Rat	Human	Rat
Porcine galanin	9.63 ± 0.07	9.49 ± 0.06	9.02 ± 0.04	8.98 ± 0.10	8.01 ± 0.06	8.14 ± 0.08
Rat galanin	9.54 ± 0.08	9.47 ± 0.11	8.79 ± 0.05	8.87 ± 0.12	7.91 ± 0.10	8.48 ± 0.18
Human galanin	9.36 ± 0.04	9.22 ± 0.09	8.63 ± 0.03	8.60 ± 0.10	7.16 ± 0.06	7.28 ± 0.04
Rat galanin-2–29	–	7.90	–	8.72	–	7.07
Porcine galanin-2–29	7.58 ± 0.05	–	8.54 ± 0.07	–	7.22 ± 0.06	–
Porcine D-Trp^2-galanin	6.20 ± 0.04	6.39 ± 0.09	6.70 ± 0.06	7.56 ± 0.13	< 6	< 6
Porcine galanin-(−7)–29	8.17 ± 0.12	7.61 ± 0.03	8.31 ± 0.03	8.60 ± 0.08	7.45 ± 0.10	7.52 ± 0.06
Porcine galanin-1–16	8.54 ± 0.14	8.70 ± 0.20	8.27 ± 0.05	8.56 ± 0.10	6.50 ± 0.06	7.05 ± 0.11
Porcine galanin-3–29	< 6	< 6	< 6	< 6	< 6	< 6
M15	9.61 ± 0.13	9.19 ± 0.13	8.97 ± 0.13	9.00 ± 0.14	7.40 ± 0.06	7.98 ± 0.06
C7	9.59 ± 0.11	9.55 ± 0.05	9.20 ± 0.03	9.25 ± 0.04	8.09 ± 0.07	8.35 ± 0.18
M40	8.62 ± 0.12	8.17 ± 0.11	8.39 ± 0.07	8.45 ± 0.16	6.54 ± 0.09	7.10 ± 0.07
M35	9.95 ± 0.11	9.49 ± 0.11	8.71 ± 0.09	8.49 ± 0.16	7.84 ± 0.08	8.68 ± 0.06
M32	9.58 ± 0.02	9.17 ± 0.13	8.84 ± 0.05	9.10 ± 0.16	8.22 ± 0.11	8.91 ± 0.06

[a]With the exception of the data obtained for rat galanin-2–29 binding to rat galanin receptors, taken from Ref. 65, all data obtained for rat galanin receptors and for human gal3 receptors are taken from Ref. 51. Human GAL1 and gal2 data are from Ref. 52.

channel activation[52,53]. In addition, gal2 can inhibit cAMP accumulation, depending on (among other things) the host cell or the G-protein repetoire in the cell, and the receptor species homologue[54,60]. For example, both Smith and co-workers and Wang and co-workers observed a galanin-dependent stimulation of inositol phosphate accumulation in Chinese hamster ovary (CHO) cells stably transfected with rat gal2, but only the cells studied by Wang and co-workers provided evidence that gal2 can also mediate a weak pertussis-toxin-resistant reduction of forskolin-stimulated cAMP, presumably through $G_{i/o}$-type G proteins[53,54]. Using HEK293 cells as the host, Fathi *et al.* reported that stable transfection of human gal2 results in a galanin-dependent increase in inositol phosphate accumulation and Ca^{2+} mobilization and a decrease in cAMP (with only the latter blocked by pertussis toxin), whereas stable transfection of rat gal2 into the same host results in galanin-dependent regulation of inositol phosphates and Ca^{2+} but not of cAMP (Refs 60, 63). Furthermore, rat gal2 was shown to stimulate MAP kinase in CHO cells; this effect is blocked by pertussis toxin or the protein kinase C inhibitor bis[indolylmaleimide], or by protein kinase C depletion, but not by expression of the β-adrenoceptor kinase C-terminal peptide, consistent with G_q-mediated signalling[54]. By contrast, rat GAL1-dependent MAP kinase activation in CHO cells is blocked by pertussis toxin and also by the β-adrenoceptor kinase C-terminal peptide, but not by bis[indolylmaleimide] or protein kinase C depletion, consistent with Gβγ release from $G_i\alpha$ (Ref. 54). Thus, gal2 could act in some cases through $G_{q/11}$-type G proteins to transmit the positive effects of galanin on Ca^{2+} flux and related events such as those observed in RIN-m5f cells (Ref. 19), pancreatic islets[15], the anterior pituitary[7] and other cells[3], with stimulatory effects on exocytosis; gal2 could act in other cases through $G_{i/o}$-type G proteins to regulate many of the processes outlined for GAL1, and to inhibit exocytosis.

Peptide analogues stimulate gal2-dependent hydrolysis of inositol phosphate release in the following order of potency: porcine galanin, porcine galanin-2–29, porcine galanin-1–16 ≫ porcine galanin-3–29; the chimeric peptides M15, M32, M35, M40 and C7 appear to be full agonists[44,53,63]. Although functional studies support the existence of native galanin receptors positively coupled to inositol phosphate hydrolysis[14,15,67], a native receptor with a well-defined gal2-like pharmacology has not yet been described. The widespread distribution of rat gal2 mRNA (Refs 47, 53, 57, 58, 63) suggests numerous physiological consequences of gal2 signalling, including prolactin release, lactation, growth hormone release, feeding, emotion, memory, nociception, cellular growth, nerve regeneration, pancreatic islet function, cardiovascular tone, peripheral metabolism and reproduction. Interestingly, the chromosome containing the gene encoding human gal2 (17q25) is associated with two human diseases (hereditary neuralgic amyotrophy and Russel–Silver syndrome), which are characterized in part by short stature and low birth weight dwarfism, respectively, in addition to developmental defects[60]; it would be useful to learn whether gal2 is involved in these disorders. In Alzheimer's disease, galanin-containing neuronal fibres hyperinnervate acetylcholine-containing neurones of the basal forebrain[68], prompting speculation that a receptor such as gal2 might function to promote survival of the dwindling

population of acetylcholine-containing neurones. In a model of experimental injury involving facial nerve crush, galanin and gal2 mRNA (but not GAL1 mRNA) are significantly elevated in motor neurones of the ipsilateral facial nucleus after nerve crush; gal2 mRNA levels peak at seven days and return to baseline within 14 days[48]. In addition, gal2 mRNA is upregulated in the rat dorsal root ganglia following inflammation, peaking after three days[47]. In both models, gal2 might promote survival and could also function as an autoreceptor. By contrast, gal2 mRNA (like GAL1 mRNA) is downregulated after axotomy, when galanin expression is dramatically upregulated[47], leaving open the possibility that other galanin receptor subtypes might function in dorsal root ganglia after axotomy.

The gal3 receptor

A third cloned galanin receptor subtype gal3 was first cloned from rat and described in two separate reports[51,65]; the sequences described in these papers diverge in four positions for reasons that are unclear, at present. Rat gal3 cDNA encodes a protein of 370 residues (Fig. 2) with an amino acid similarity to rat GAL1 of 36% and to rat gal2 of 55%. The sequence similarity to gal2 is higher within TM2–TM4, in which amino acid similarities range from 70% to >90% (Fig. 2). There are 83 amino acids conserved in all three rat galanin receptor subtypes, yielding a shared amino acid similarity of ~23%. Although the galanin receptor proteins are similar (30–38%) to the somatostatin sst_4 and sst_5 receptor subtypes and the ORL1 (nociceptin) receptor, as well as the rat GPR54 receptor (37%), they form a distinct subfamily[38,51]. Human gal3 was cloned from a human genomic library based on structural similarity to human GAL1 and gal2, and found to contain an intron in the same location (after TM3) as that described for gal2 (Ref. 51). It remains to be determined whether a *PstI* site polymorphism, recently identified in the human gal3 intron, has physiological significance[69]. Human gal3 protein contains 368 amino acids and 90% similarity to rat gal3. Both human and rat gal3 homologues contain a single consensus site for N-linked glycosylation, and multiple intracellular consensus sites for phosphorylation. Each contains a consensus site for protein kinase C phosphorylation in the C-terminal tail; however, this site is not conserved, which suggests the possible existence of distinct regulatory mechanisms. The human gal3 was mapped to 22q12.2–13.1 (Ref. 70). A mouse gal3 homologue was cloned and mapped to mouse chromosome 15 (Ref. 62).

Transcripts of gal3 were first reported, using northern blot analysis, in the heart, spleen and testes[65]. Using the more sensitive method of RNase protection, Smith and co-workers detected the highest abundance of rat gal3 transcripts in the hypothalamus and pituitary; they also found gal3 distributed among discrete regions of the rat CNS such as the olfactory bulb, cerebral cortex, medulla oblongata, caudate putamen, cerebellum and spinal cord but not in the hippocampus or substantia nigra. Rat gal3 transcripts have also been detected in peripheral tissues including the liver, kidney, stomach, testicles, adrenal cortex, lung, adrenal medulla, spleen and pancreas but not in the heart, uterus, vas deferens, choroid plexus or dorsal root ganglia[51].

The pharmacology of gal3 combines elements of GAL1 and gal2 pharmacology (Table 1). Both GAL1 and gal3 display a low affinity for M40 compared with their affinity for other chimeric peptides[51,65]. Both gal2 and gal3 display less than tenfold selectivity for binding porcine galanin compared with galanin-2–29, whereas GAL1 displays >100-fold selectivity for porcine galanin[65]. All three subtypes have greater affinity for the conserved N-terminal portion of galanin than for other compounds tested and have no affinity for galanin-3–29. Clearly, chemically diverse ligands that are selective for the receptor subtypes are needed to develop more definitive pharmacological profiles.

Human and rat gal3 share similar pharmacological profiles in [^{125}I]galanin receptor binding assays (Table 1). Furthermore, they both stimulate a pertussis-toxin-sensitive activation of an inward K^+ current when transfected into *Xenopus* oocytes with the G-protein-coupled, inwardly rectifying K^+ channels 1 and 4, which is consistent with coupling to $G_{i/o}$-type G proteins[51]. Chimeric peptides (C7, M15, M32, M35 and M40) are agonists in the assay using G-protein-coupled, inwardly rectifying K^+ channels and human gal3; M40 displays the lowest potency[51]. Thus, gal3, like GAL1, is capable of producing a hyperpolarizing response consistent with the inhibition of exocytosis. A native cell line or tissue model with a distinctive gal3-like pharmacology has not yet been described. However, localization of gal3 mRNA in regions such as the caudate putamen, hypothalamus, pituitary, spinal cord, pancreas, liver, kidney, stomach and adrenal gland[51] suggests that gal3 might be involved in emotion, feeding, pituitary hormone release, nociception and metabolism. Considering the expression of gal3 mRNA in the pancreas, hyperpolarizing capabilities of gal3 and weak interactions of gal3 with M40, it is feasible that gal3, like GAL1, regulates insulin and glucose homeostasis[26,51].

In vivo responses to galanin: comparison with cloned receptors

The effects of galanin *in vivo* have been studied extensively[3,5]; experimental paradigms include modulation of acetylcholine release, memory and Alzheimer's disease[71,72], feeding behaviour[73], pain[3], depression[3], gut secretion and motility[74], cardiovascular tone[75], cerebrovascular tone[76], neuroendocrine regulation, lactation and reproduction[77] and neuroregeneration[78]. An in-depth discussion of these effects is beyond the scope of this review; the few models selected here are not comprehensive but are intended to point out discrepancies with the cloned receptor pharmacology.

Galanin as a stimulant of food intake

Much interest has centred on galanin as a stimulant of food intake in rats. Injection of galanin into the intracerebral ventricles, the paraventricular nucleus of the hypothalamus or the amygdala produces a dose-dependent increase in food intake in rats, which can be blocked by C7 and M40 (Refs 33, 73, 79, 80). Although all three cloned galanin receptor subtypes are present in brain regions important for galanin-stimulated feeding[40,41,51,63], and GAL1 mRNA is upregulated by inhibitors of glucose and fat metabolism[49], it is difficult to assign

these feeding responses conclusively to a single subtype, particularly because the chimeric peptides C7 and M40 appear to be full agonists for the cloned receptors[9,51,52,60] (although see concluding remarks). The rat feeding response was attributed, by Wang *et al.*, to either GAL1 or an unknown galanin receptor subtype rather than to gal2 and gal3; the authors based this hypothesis on the ability of galanin to produce a greater increase in feeding than galanin-2–29, galanin-3–29 or galanin-1–16 following intracerebroventricular administration. However, this analysis is limited by the omission of dose–response curves for chimeric peptides such as M40 and C7 (Ref. 81). It is interesting to note that, in the same report, Wang *et al.* propose that gal2 is involved in jejunal contraction because of the abundance of gal2 mRNA in the jejunum, and the relative strength of contraction induced by galanin-2–29 and galanin-1–16 compared with galanin and galanin-3–29.

Galanin-induced impairment of memory

Another physiological response to galanin that is of interest is its ability to impair performance in a delayed 'non-matching to position' memory task when applied to the lateral ventricles or hippocampus[25]. These effects are also blocked by M40. Such observations, coupled with the demonstration that galanin inhibits the release of acetylcholine[82], has led to speculation that a galanin receptor antagonist might enhance cognition. However, galanin does not impair performance in every paradigm, exhibiting dose-dependent biphasic effects on acquisition and retention after bilateral injection into the rat ventral hippocampus, for example[83], and showing no effect on acquisition in at least one study based on the Morris water maze task[84]. By contrast, the chimeric peptide M35 is reported to facilitate acquisition in the Morris water maze model[85]. Despite this, further interest in galanin and cognition has been sparked by descriptions of extensive galaninergic neuronal hyperinnervation of the basal forebrain in patients with Alzheimer's disease[68]. In a recent report of rats that possess lesions of acetylcholine-containing regions of the basal forebrain, a combination of M40 plus the M_1 muscarinic acetylcholine receptor agonist 3-(3-*S*-*n*-pentyl-1,2,5-thiadiazol-4-yl)-1,2;5,6-tetrahydro-1-methylpyridine (TZTP) was shown to improve delayed 'non-matching to position' choice accuracy[86]. Again, it is difficult to reconcile the effects of galanin *in vivo* with any single cloned receptor subtype because of the disparity of the activity of M40 as an antagonist of the galanin response *in vivo* compared with its agonist properties in cloned receptor assays. Further characterization of this paradigm using reliable pharmacological tools (i.e. stable, selective and potent antagonists of GAL1, gal2 and gal3) would help to clarify the relationships.

Galanin and pain

A third area of interest in the physiology of galanin is antinociception. Galanin has been studied in several experimental models of pain. In the rat flexor reflex model, which is a measure of motor response to nociceptive stimuli, intrathecally applied galanin facilitates the nociceptive reflex at a low dose but inhibits it at a high dose[87]. This facilitation is blocked by C7 and M32, whereas M40 is a partial receptor agonist[32]; this is not easily reconciled with a cloned receptor profile. In the tail flick and Randall–Selitto tests, intrathecal application of galanin potentiates the effect of morphine-induced analgesia, whereas opposite effects are produced by M35 and galantide[88]. Again, the failure to detect any antagonist behaviour in cloned receptor assays cannot be reconciled easily with the effects of these same chimeric peptides *in vivo*. A role for GAL1 in the nociceptive reflex pathway was suggested after it was shown that intrathecal administration of a cell-penetrating peptide nucleic acid complementary to the GAL1 blocked the inhibitory effect of galanin on the flexor reflex in the rat; however, the effect on the activity of chimeric peptides such as M40 and C7 was not reported[89]. Interestingly, after sciatic nerve transection, galanin and galanin message-associated protein (GMAP) undergo a dramatic and long-lasting upregulation in dorsal root ganglia[90,91]. Administration of galanin antisense oligonucleotides to the transected sciatic nerve suppresses the expression of galanin and also the development of autotomy that typically accompanies a sciatic nerve cut[92]. In transgenic mice from which the gene encoding galanin had been knocked out, distinguishing features included a loss of galanin-containing neurones in dorsal root ganglia, a defective rate of nerve regeneration and the absence of autotomy after a sciatic nerve cut[93], in addition to defects in prolactin secretion and lactotroph function[94]. Electrophysiological studies of C-type and A-type neurones from the dorsal root ganglia of rats support the appearance of a novel, galanin-dependent, inward membrane current of undefined ionic composition after axotomy[95]; the effect of axotomy on gal3 mRNA, normally undetectable in dorsal root ganglia[51], is not yet known. These morphological and physiological studies suggest that galanin receptors might be active in hyperalgesic states such as neuropathological pain but the roles of individual subtypes remain to be determined.

Concluding remarks

It is clear that there is a lack of concordance of profiles for cloned galanin receptor subtypes with the effects of galanin-like peptides in native systems. The cloned receptors do not readily account for the reported antagonist actions of the chimeric peptides M15, M32, M35, C7 and especially M40, nor do they account for responses to galanin-3–29. Furthermore, the cloned receptors do not account for the enhanced potency or opposing actions of galanin-1–15 compared with full-length galanin. There are several possible interpretations of these discrepancies. First, it cannot be excluded that additional receptor subtypes, such as galanin-3–29- or galanin-1–15-preferring subtypes, have yet to be cloned. Second, it might be that the peptide tools used for evaluating these receptors thus far are chemically or biologically unstable, or otherwise sensitive to diverse environmental effects when applied to preparations *in vivo* or *in vitro*. Third, it is possible, although unlikely, that the lack of detectable antagonist activity in the heterologous expression systems is artifactual, as a result of high levels of receptor reserve. Work by Fitzgerald and co-workers[55] argues against this possibility for the activity of M40 at human GAL1 in HEK293 cells. Furthermore, [^{125}I]M40 binding to rat brain sections was reduced by GTPγS in an agonist-like fashion throughout the brain[96].

Workers in the field of galanin research await the discovery of low molecular weight receptor antagonists (and agonists) that could clarify these relationships and help to define more clearly the roles of the cloned galanin receptors *in vivo*. The only non-peptide compound reported to bind to galanin receptors is the fungal metabolite SCH202596, a spitocoumaranone with a molecular mass of 353 Da (Ref. 97). This compound binds to human Bowes melanoma cell membranes with an IC_{50} of 1.7 μM. It is expected that additional leads with affinity and selectivity for each of the galanin receptor subtypes will emerge eventually. Along the way, transgenic knockout animals, antisense techniques, orphan receptor characterization and continued cloning efforts might provide early clues to the roles of galanin and its receptors in physiology and pathophysiology.

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Chemical names

M15: galanin-1–13-substance-*P*-5–11 amide, also known as galantide

M32: galanin-1–13-NPY_{25-36} amide

M35: galanin-1–13-bradykinin-2–9 amide

M40: galanin-1–13-Pro-Pro-Ala-Leu-Ala-Leu-Ala amide

C7: galanin-1–13-spantide amide, substance P receptor antagonist

SCH202596: 2-[(5-methoxy-3-oxo-1-carboxymethyl)-6-spiro(1,4-cyclohexadienyl)]-(3*S*,4*S*,5*S*,6*S*)-6-[(5,7-dichloro-6-methyl-3-oxo-2,3-dihydrobenzo[*b*]furan-4-yl)oxyl]-3,4,5-trihydroxy-1-cyclohexene-1-methylcarboxylate

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